KERNFORSCHUNGSZENTRUM

KARLSRUHE

Mai 1963

KFK 173

Institut für Strahlenbiologie

Dose-Dependence of the Frequency of Radiation-Induced Recessive Sex-Linked Lethals in Drosophila Melanogaster, with Special Consideration of the Stage Sensitivity of the Irradiated Germ Cells

H. Traut

KERNREAKTOR

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KARLSRUHE

Reprinted from Sobels: "Repair from Genetic Radiation"
PERGAMON PRESS
OXFORD · LONDON · NEW YORK · PARIS
1963

DOSE-DEPENDENCE OF THE FREQUENCY OF RADIATION-INDUCED RECESSIVE SEX-LINKED LETHALS IN *DROSOPHILA MELANOGASTER*, WITH SPECIAL CONSIDERATION OF THE STAGE SENSITIVITY OF THE IRRADIATED GERM CELLS

H. TRAUT
Institut für Strahlenbiologie, Kernforschungszentrum Karlsruhe, Germany

INTRODUCTION

In recent years, the relationship between the frequency of radiation-induced mutations in different stages of the developing male germ cells of *Drosophila* has received considerable attention (see Muller, 1959). Prior to these studies a great number of investigations had been carried out on the dose-dependence of the induced mutation frequency. Recessive sex-linked lethals, in particular, were the object of these investigations (see Timoféeff-Ressovsky and Zimmer, 1947). Since, however, the importance of stage sensitivity was not yet fully realized at that time, insufficient consideration was given to this variable. A detailed study of both parameters together, dose-dependence and stage sensitivity, has hitherto not been carried out, possibly because of the time-consuming procedures involved in such experiments.

Since an analysis of the dose-effect relationship for stages with different radiosensitivity might help to understand the mechanism of the radiation-induced mutation process, it was thought that such an investigation would be of value. Moreover, Muller *et al.* (1954) have pointed out that results from earlier work in the relation between mutation rate and radiation dose should be considered with caution, because these experiments have not yet been repeated with methods which are now available.

These reasons prompted us to a renewed study of the dose-effect relationship for mutations induced in stages with different sensitivity to X-irradiation. The successive stages of germ cell development were sampled by means of the well-known brood pattern technique. The genetic effects investigated were recessive sex-linked lethals, autosomal translocations and sex-chromosome loss. In this context only the results with lethals will be reported.

METHODS

Our experiments comprise 3 series. We irradiated:

(1) 3–4-day-old adult males,

(2) 174-hr-old male pupae,*

(3) Sperm, stored in inseminated females.

All work was done with the dual-purpose strain constructed by Oster (1958a, b),† which permits testing for both sex-linked lethals and autosomal translocations from the progeny of the *same* irradiated males. The genotype of the P-males was B, that of the P-females y sc^{81}In-49 sc^{8}; bw; st. For consideration of stage sensitivity the males were mated every $24 \pm \frac{1}{2}$ hr with fresh virgins in series (1) and (immediately after the hatching) in series (2). With this procedure the ratio of P-females to P-males was 10:1 for the first three one-day-broods in series (1), and 5:1 for the following broods and for series (2). As has been shown by Mossige (1955) and Traut (1960), the excess of females appears necessary to achieve maximum utilization of sperm.

In series (3) the lethal rates are based on progeny sampled during 16 days following exposure to irradiation (4 4-day periods). The temperature was maintained at 25 ± 1 °C throughout. The flies were irradiated with 150 kV X-rays at a dose rate of 500 r/min (current: 15 mA). The h.v.l. was 6 mm Al.‡

RESULTS

With respect to *stage sensitivity* our results with adult males agree with those of other workers using a refined brood pattern technique (e.g. Mossige 1956). Characteristic brood patterns are shown in Fig. 1. The existence of the postmeiotic sensitivity minimum (day $2 + 3$ after radiation exposure) is demonstrated in Fig. 2. The experiments with 174 hr old pupae show that, as had been observed before by Khishin (1955) and Oster (1958a, b), germ cells of this pupal stage are characterized by a relatively high sensitivity (Fig. 3). The mutation rate of sperm irradiated in females did not change appreciably within 4 4-day periods of egg-laying after irradiation. Data are given in Tables 1 and 2 for adult males and pupae respectively, and in Table 3 for inseminated females. Since we would prefer to discuss the problems of stage sensitivity only with regard to the question of dose-relationship, a detailed discussion of differential radiosensitivity will appear elsewhere.

With respect to *dose-dependence*, statistically significant departures from linearity were obtained. In most cases, the mutation rates are relatively low, not much exceeding 10 per cent. Consequently, the fact that, in accordance with the Poisson-distribution of hit events, more than one mutation may be

* The experiments with pupae were done in collaboration with W. Ebeling (Karlsruhe).

† We are indebted to Dr. I. I. Oster (Philadelphia) for providing this strain.

‡ We are grateful to Dr. A. Müller and W. Köhnlein (Karlsruhe) for their advice and help in dosimetry.

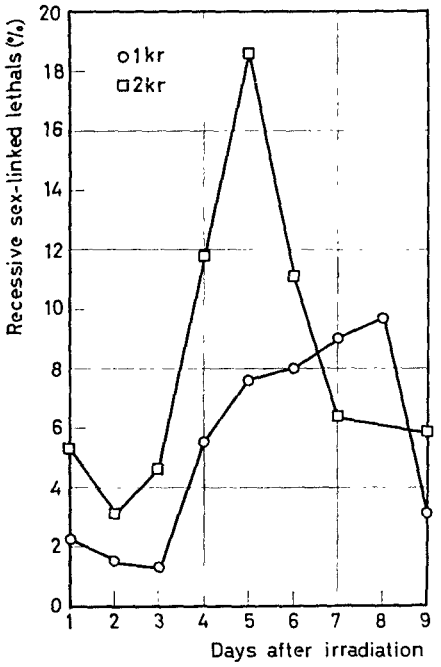


FIG. 1. (*left*) The dependence of radiation-induced lethal rate on stage sensitivity after irradiation of 3–4-day-old B-males; 9 one-day broods. Spontaneous rate subtracted. For the supporting experimental data see Table 1.

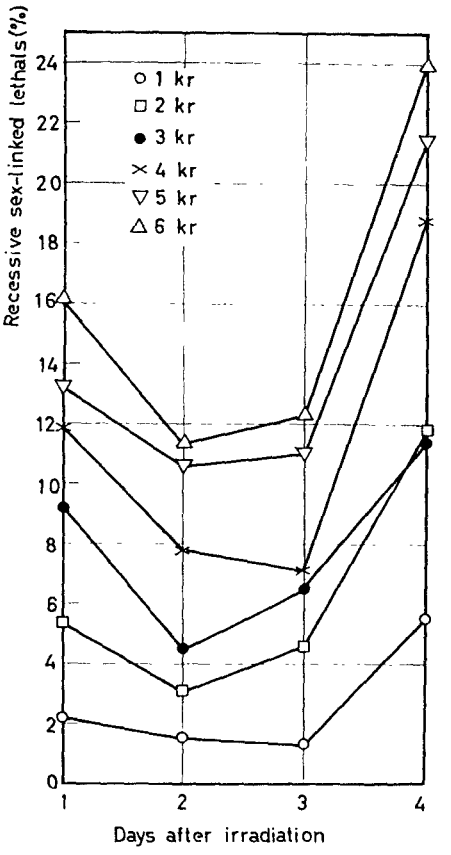


FIG. 2. (*right*) The dependence of radiation-induced lethal rate on stage sensitivity after irradiation of 3–4-day-old B-males with different doses; 4 one-day broods. Spontaneous rate subtracted. For the supporting experimental data see Table 1.

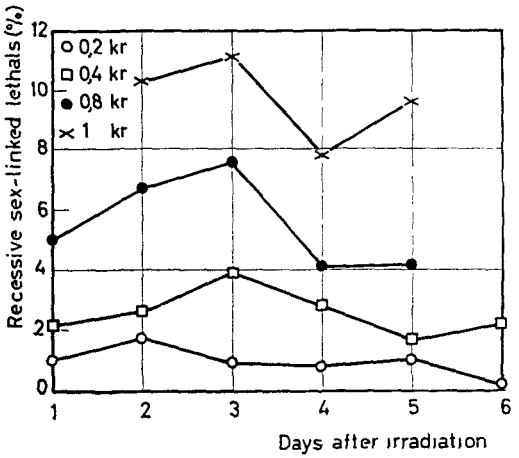


FIG. 3. The dependence of radiation-induced lethal rate on stage sensitivity after exposure of male 174-hr-old B-pupae to different doses of X-irradiation; 6 one-day broods. Spontaneous rate subtracted. For the supporting experimental data see Table 2.

TABLE 1. THE DEPENDENCE OF LETHAL RATE ON RADIATION DOSE AND STAGE SENSITIVITY. IRRADIATION OF 3–4-DAY-OLD B-MALES. SPONTANEOUS RATE: 0·42 PER CENT (22/5296), BASED ON SPERM OF THE FIRST 9 DAYS

Dose (r)	Day 1	Day 2	Day 3	Day 4
1000	2·6% (119/4511)	1·9% (66/3443)	1·7% (52/3034)	5·9% (137/2338)
2000	5·7% (206/3624)	3·5% (85/2412)	5·0% (96/1929)	12·2% (87/715)
3000	9·6% (301/3154)	4·9% (131/2682)	6·9% (137/1987)	11·8% (87/735)
4000	12·3% (207/1683)	8·2% (149/1812)	7·5% (143/1904)	19·2% (62/322)
5000	13·6% (232/1702)	11·0% (194/1766)	11·4% (154/1350)	21·9% (39/178)
6000	16·5% (102/618)	11·7% (178/1516)	12·7% (120/942)	24·4% (34/139)

TABLE 2. THE DEPENDENCE OF LETHAL RATE ON RADIATION DOSE AND STAGE SENSITIVITY. IRRADIATION OF 174 HR-OLD MALE B-PUPAE. SPONTANEOUS RATE: 0·61 PER CENT (19/3094), BASED ON SPERM OF THE FIRST 6 DAYS AFTER HATCHING OF THE MALES

Dose (r)	Day 1	Day 2	Day 3
200	1·6% (34/2109)	2·3% (37/1584)	1·5% (17/1100)
400	2·7% (56/2071)	3·2% (34/1054)	4·5% (54/1191)
800	5·6% (59/1063)	7·3% (106/1459)	8·1% (98/1210)
1000	—	10·9% (106/968)	11·7% (55/471)

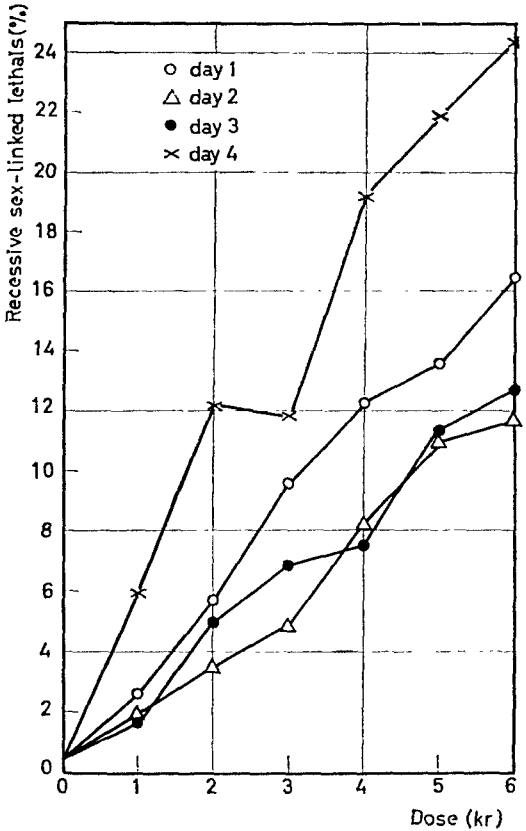


FIG. 4. (*left*) Dose effect curves for lethals induced in stages with different sensitivity. 3–4-day-old B-males were irradiated. For the supporting experimental data see Table 1.

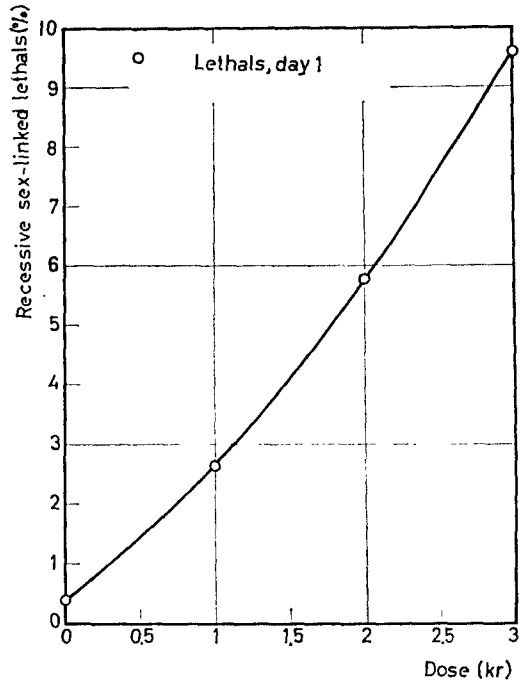
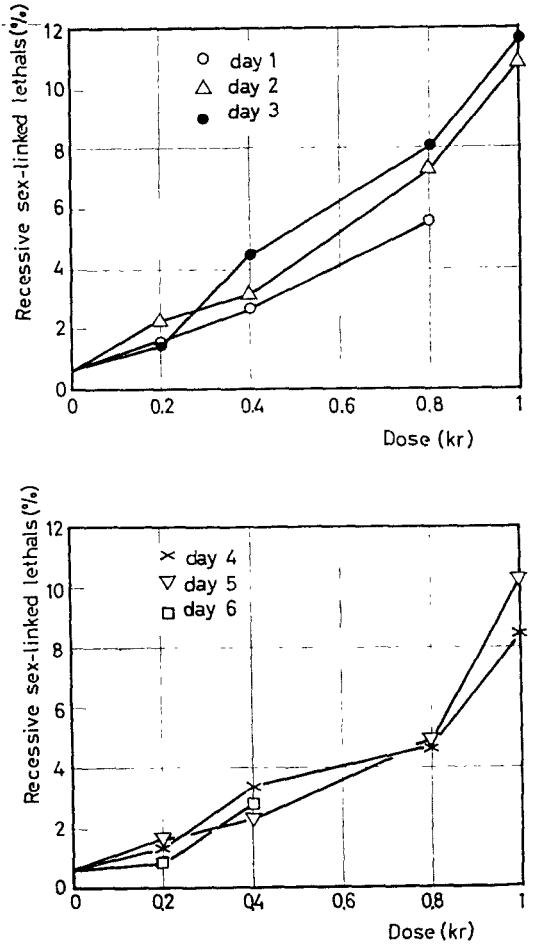


FIG. 5. The lethal rate of the first day after irradiation (0–3 kr, see Fig. 4). The fitted equation is: $y = 0{\cdot}42 + 1{\cdot}80x + 0{\cdot}42x^2$ (y = lethal rate in per cent, x = dose in kr).

second day curve increases faster than dose ($P < 0{\cdot}01$) up to 5 kr, but shows a flattening at high doses (5–6 kr). The third and fourth day curves are characterized by a stepwise increase of lethal rate with dose. From the experiments with male *pupae* (Figs. 6 and 7, Table 2) we obtained dose-exponents significantly greater than one for the second, fifth and sixth day,



FIGS. 6 and 7. The dose-dependence of the radiation-induced lethal rate, considering stage sensitivity. 174 hr-old male B-pupae were irradiated. For the supporting experimental data see Table 2.

while the deviations from linearity of the first, third, and fourth days are not significant. *For sperm irradiated in females* (Fig. 8, Table 3) no "steps" are formed. Here the rise above linearity, after a linear increase up to 4 kr, is

TABLE 2. (*continued*)

Dose (r)	Day 4	Day 5	Day 6
200	1·4% (21/1470)	1·6% (38/2390)	0·8% (11/1316)
400	3·4% (33/982)	2·3% (53/2292)	2·8% (27/962)
800	4·7% (37/791)	4·8% (20/419)	—
1000	8·4% (33/391)	10·2% (40/392)	—

TABLE 3. THE DOSE-DEPENDENCE OF THE RADIATION-INDUCED LETHAL RATE FOR SPERM IRRADIATED IN FEMALES. PROGENY OF THE FIRST 16 DAYS FOLLOWING IRRADIATION WAS TESTED. THE SAME SPONTANEOUS RATE IS USED AS IN TABLE 1 (P-MALES WERE 3–5, P-FEMALES $\geqslant$ 3 DAYS OLD)

Dose (r)	Lethals
0	0·42% (22/5296)
200	0·91% (49/5384)
500	1·9%(148/7979)
1000	3·6% (137/3845)
1500	5·5% (422/7706)
2000	6·8% (366/5376)
3000	9·6% (226/2351)
4000	13·5% (291/2158)
5000	24·2% (39/161)

registered as one event, is not taken into consideration in the statistical tests. These tests are based on a specified analysis of variance applied to weighted regressions (see Quenouille, 1950 and Armitage, 1955).* The departures from linearity consist (1) in dose-exponents significantly greater than one and (2) in the formation of "steps". The latter effect is difficult to treat statistically, but it would seem that the stepwise nature of some of our dose-effect curves is correlated with significant terms greater than one of the regressions. In the experiments with *adult males* (Fig. 4, Table 1), dose-effect curves from the first 4 days after irradiation were obtained; the marked decrease in the number of progeny did not permit such an analysis for later days than the fourth to be made. The lethal rate for the first and second day increases with a dose-exponent significantly greater than one. For the first day this dose-dependence is found only in the range from 0 to 3 kr (P for deviation from linearity $<0{\cdot}01$; see Fig. 5); with higher doses (3–6 kr) the curve is "stepped". The

* We are grateful to professors Dr. A. Catsch (Karlsruhe) and Dr. G. Koller (Wiesbaden) for their advice in the statistical analysis. The calculations were performed with an electronic computer with the kind help of Dr. W. Häfele, Dr. J. Merkwitz, Dipl. Math.H. Kraetsch and Miss B. Zschiesche (Karlsruhe).

evident, though the value for 5 kr is based on only a small number of chromosomes tested. Because of the induction of dominant lethals in sperm as well as in oocytes, it is difficult to obtain sufficient material for higher doses when the progeny of sperm irradiated in females is tested. Nevertheless further experiments with high doses are under way.

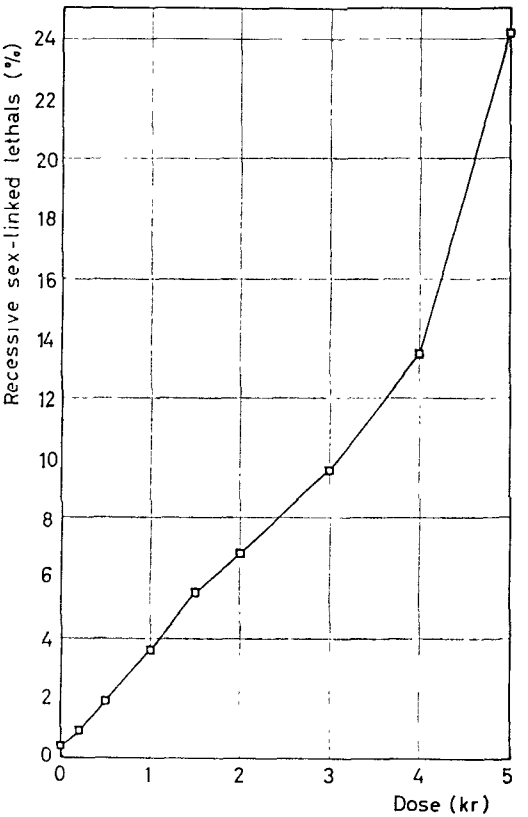


FIG. 8. The dose-dependence of the radiation-induced lethal rate for sperm irradiated in females. For the supporting experimental data see Table 3.

DISCUSSION

The departures from linearity as observed in our experiments with adult males, male pupae and inseminated females may be discussed in the following way.

First, the deviations could have originated from selection among F_1 flies. This possibility has been extensively discussed elsewhere (Traut, 1962). It is thought unlikely that this factor is the main cause for the observed deviations. Here, the more probable possibility—delayed sperm release—will be considered again. By this we mean that sperm which is mature at a definite day

after irradiation is not utilized until one or more days later. Although this possibility cannot be ruled out, we do not believe that this factor has contributed significantly to the departures from linearity observed. For the main cause of delayed sperm release, an insufficient number of P-females per P-male per day, has been eliminated by using a large excess of virgin P-females. Moreover, in order to avoid affecting the mating behaviour by mould or bacterial infections, etc., the condition of the culture bottles in which the P-flies were mated, was checked daily. Finally, I want to emphasize that the biological conditions in our experiments were kept constant as far as possible for each radiation dose. We feel, therefore, that it is reasonable to discuss other more interesting aspects of our results.

The *dose-exponents greater than one* could be interpreted by assuming a two-hit component in the total lethal rate. These two-hit lethals might be due to either position effect as, for example, inversions and translocations, or multibreak deficiencies. This has a bearing on the "mutation-by-breakage hypothesis" put forward by Lea and Catcheside 20 years ago (see Lea, 1946). This hypothesis sought to explain the discrepancy between the "classic" linear dose-relationship for sex-linked lethals and the relatively high contribution by two-hit chromosome aberrations to the total lethal rate. The existence of recessive lethals based on position effect, although not valid under the mutation-by-breakage hypothesis, is confirmed by our finding of dose exponents greater than one. Our results agree, however, with the supposition of Muller (1954, 1957, 1959) that in the "classic" linear dose-relationship, the tendency of the mutation frequency to increase faster than dose in consequence of position effect lethals, is more or less compensated by the "lowering" effect of other factors. In addition, some of the two-hit lethals may have resulted from induction of two so-called semilethals, each of which alone does not suffice to act as a lethal, but by acting together would produce the same effect as one regular recessive lethal. However, according to the results of Ives (1959a), semilethals are induced too infrequently to be of statistical importance. Dose-exponents greater than one for the induction of visibles by ionizing radiation have been reported by several authors: for the mould *Aspergillus* by Stapleton *et al.* (1952), for the wasp *Mormoniella* by Kayhart (1956) and for *Drosophila* by Ives (1959b). For radiation-induced recessive sex-linked lethals in *Drosophila* oocytes this effect is reported by Parker (1960). The findings of Edington (1956) and Edington *et al.* (1962) concerning male germ cells of *Drosophila* are contradictory: that is, if the P-males were discarded after 6 days, a dose exponent greater than one was obtained for X-rays,* but not for gamma-rays. In contrast with our results, however, no significant departures from linearity were obtained, if only sperm of the first day after irradiation was tested, although there is a tendency

* These experimental conditions correspond to those of earlier investigations, leading to a linear relationship (see e. g. Timoféeff-Ressovsky and Zimmer, 1947).

in the expected direction (rise above linearity). It is of interest that Edington *et al.* (1962) found an exponent greater than one for the kind of lethals which are ordinarily suppressed by the Y-chromosome and are only detectable by a modified technique. The fact (Lindsley *et al.*, 1960), that part of the Y-suppressed lethals originate from chromosome aberrations, which are likely to act *via* position effect, agrees very well with this finding.

The "*steps*" of our dose-effect curves can be explained on the following two assumptions: (1) Different sensitivities of germ cells even within one-day broods. (2) A positive correlation between the sensitivity of chromosomes to the induction of mutability and dominant lethality. If the latter case were true one would, with increasing dose, expect a successive elimination of the more sensitive germ cells through the production of dominant lethals. It can be shown mathematically that by some simple assumptions (Dittrich, personal communication) such an elimination can indeed lead to the formation of a "step". Muller *et al.* (1954) have proposed such a selection mechanism to interpret their finding of a marked flattening of the dose-effect curve for lethals at high dosage after the irradiation of heterogeneous germ cell samples in *Drosophila*. Similar results were obtained by Sobels and Tates (1961). In this context I want to point out that very clear steps were also obtained in our data on X-chromosome loss after irradiation of *Drosophila*-oocytes (oviposition restricted to the first 3 days after irradiation). The same interpretation seems to hold for this situation.

Our results, in consequence, agree with Muller's supposition (1954, 1957, 1959) that the "classic" linear dose function for recessive sex-linked lethals, especially at relatively high doses, results from at least two counteracting factors: (1) The influence of a two-hit component, the relative contribution of which to the total frequency increases with dose, resulting in dose-exponents greater than one in relatively homogeneous material. (2) The heterogeneity of the irradiated material with respect to radiation sensitivity (see Fig. 2), leading to a flattening of dose-effect curves. Our finding of dose-exponents greater than one for germ cell material which is made relatively homogeneous points to the operation of factor (1). Evidence for the operation of factor (2) is inferred from the "stepwise" increase of lethal rate with dose. This is taken as an indication that even in 1-day broods, the material may be heterogeneous. The observation that in our experiments with inseminated females, where completely homogeneous germ cell material can be expected, no "steps" are obtained, is in line with the latter interpretation. These two counteracting factors may compensate each other and thus simulate a linear dose-relationship. This is supported by a comparison of our lethal data for the first 4 days after irradiation of adults with those published by Timoféeff-Ressovsky and Zimmer (1947). The agreement between the pooled data which were obtained by calculating for each dose the arithmetic mean for the lethal frequencies of the four broods, and the linear function of Timoféeff-Ressovsky

and Zimmer is rather good, that is if the 6 kr-value is ignored (Fig. 9, Table 4). I want to emphasize, however, that the calculation of arithmetic means of the mutation rates of the first 4 days only roughly approximates the

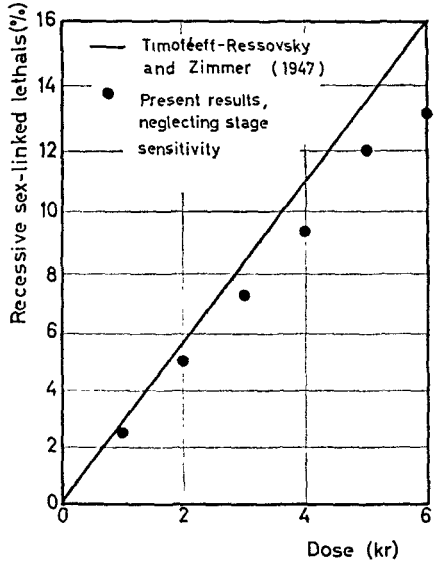


FIG. 9. The dose-dependence for the mean lethal frequencies sampled during the first four days after irradiation (see text). Irradiation of 3–4-day-old B-males. For the supporting experimental data see Table 1. Spontaneous rate subtracted. Comparison of these pooled data with the dose-effect curve, published by Timoféeff-Ressovsky and Zimmer (1947). Abscissa linear, ordinate logarithmic. The material is the same as that presented in Fig. 4 and Tables 1 and 4.

TABLE 4. THE DOSE-DEPENDENCE OF RADIATION-INDUCED LETHAL RATE, BASED ON THE FIRST 4 DAYS AFTER THE IRRADIATION OF 3–4 DAY-OLD B-MALES. SPONTANEOUS RATE: 0·42 PER CENT (SEE TABLE 1)

Brood	1 kr	2 kr	3 kr	4 kr	5 kr	6 kr
Day 1	119/4511	206/3624	301/3154	207/1683	232/1702	102/618
Day 2	66/3443	85/2412	131/2682	149/1812	194/1766	178/1516
Day 3	52/3034	96/1929	137/1987	143/1904	154/1350	120/942
Day 4	137/2338	87/715	87/735	62/322	39/178	34/139
Total	374/13326	474/8680	656/8558	561/5721	619/4996	434/3215
%	2·8	5·5	7·7	9·8	12·4	13·5
minus spontaneous rate (%)	2·4	5·1	7·3	9·4	12·0	13·1

biological conditions of the earlier experiments showing a linear relationship. Nevertheless, this comparison exemplifies the findings of Zimmer (1960) and Dittrich (1960) that dose-effect curves, even of the one-hit type, may not necessarily be the result of one-hit events only, but also of a variability in either the number of sensitive units per object which must be hit (i.e. the hit number), or the sensitivity of these units (i.e. the formal target volume). With regard to our results of irradiated 174 hr-old pupae, it can be seen that the pooled data, obtained in a similar way as those for adults, increase with a higher power of the dose than 1 (Fig. 10, Table 5). This demonstrates the

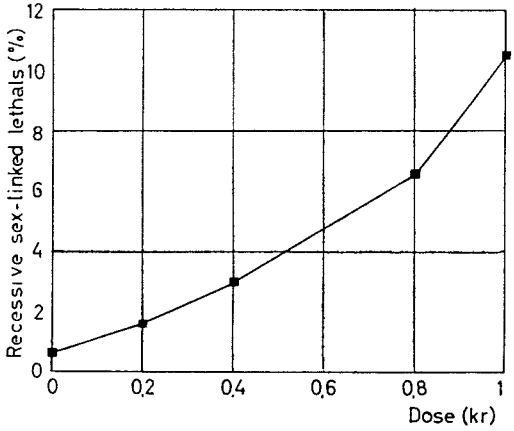


FIG. 10. The pooled data for 174-hr-old male pupae, obtained in the same way as those in Fig. 9 (see text). Spontaneous rate not subtracted. The material is the same as that presented in Figs. 6 and 7 and Tables 2 and 5.

TABLE 5. THE DOSE-DEPENDENCE OF RADIATION-INDUCED LETHAL RATE, BASED ON THE FIRST 6 DAYS AFTER THE HATCHING OF B-MALES, WHICH WERE IRRADIATED AS 174 HR-OLD PUPAE. SPONTANEOUS RATE: 0·61 PER CENT (SEE TABLE 2)

Brood	0·2 kr	0·4 kr	0·8 kr	1·0 kr
Day 1	34/2109	56/2071	59/1063	—
Day 2	37/1584	34/1054	106/1459	106/968
Day 3	17/1100	54/1191	98/1210	55/471
Day 4	21/1470	33/982	37/791	33/391
Day 5	38/2390	53/2292	20/419	40/392
Day 6	11/1316	27/962	—	—
Total	1·6% (158/9969)	3·0% (257/8552)	6·5% (320/4942)	10·5% (234/2222)

accidental nature of the linear dose-relationship for adults with high doses, when the stage sensitivity is neglected (Fig. 9). A linear relationship, on the other hand, was obtained by Oster (1958c and personal communication) after irradiation of approximately the same pupae stage with doses of 250

and 1000 r, and a mating procedure of three females per newly hatched male, during the first three days. His data, however, show a tendency, though this is not significant statistically, in the expected direction, that is an increase above linearity.

SUMMARY

In the experiments showing a linear dose-effect relationship for sex-linked recessive lethals in *Drosophila* males, insufficient consideration has been given hitherto to the dependence of radiation sensitivity on cell stage, for the latter effect came to attention only in recent years. The effect of radiation dose on mutation frequency has, therefore, been subjected to a renewal study, but in close association with differential radiosensitivity. The following results were obtained: (1) dose-exponents significantly greater than one for certain dose-ranges, (2) indication of a stepwise increase of lethal rate with dose. Most of the departures from linearity thus obtained are statistically significant. Our results have been discussed in relation to the "mutation by-breakage hypothesis" (Lea) and the problem of "position-effect lethals". They seem to confirm Muller's suggestion that at relatively high doses, the linearity of the "classical" dose-effect curve for recessive sex-linked lethals is caused more or less incidentally by the counteraction of different factors. Furthermore, our findings support the calculations by Zimmer (1960) and Dittrich (1960) showing that curves of the one-hit type may not necessarily be the result of one-hit events alone, but also, of the biological variability of the irradiated material with respect to hit number and formal target volume.

ACKNOWLEDGEMENTS

I am indebted to professors Dr. K. G. Zimmer, Dr. A. Catsch, Dr. W. Dittrich and Dr. H. Bauer for many valuable suggestions. I wish to thank Dr. I. I. Oster for having critically revised the manuscript. Prof. F. H. Sobels and Dr. J. W. Boag kindly have revised the English text. The efficient help of Miss U. Apitzsch and Miss I. Ufholz is gratefully acknowledged.

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DISCUSSION

MULLER: All I want to say is that I think he has proved his case so well that there is very little left to say.

ALPER: Perhaps Dr. Oftedal would like to introduce another step in that first range from 0–200 r.

OFTEDAL: I don't think I can elaborate that on the spur of the moment. What you are thinking of concerns the difference between Abrahamson's data and mine, which should be compatible. If there is a step there it could also be a heterogeneity within the cell cycle of the spermatogonia, whereas Traut's data refer to postmeiotic cells.

ALPER: This data is very comforting, if you account for fractionation effects in terms of what is now generally believed about fractionation effects on cell survival. It was very difficult to understand how fractionation could have a sparing effect, if there was strictly a linear dose dependence. If you have that sort of function and you can assume that you've got submutational damage corresponding to the sublethal damage represented by a shoulder in the cell survival curve and you fractionate you could presumably expect your curve to be repeated as if from zero dose. This would be consistent with fractionation effects. The latter would be very difficult to understand if you had complete linearity.

AUERBACH: These are stages in which no fractionation effects have been observed. It was observed in spermatogonia or in oogonia.

TRAUT: It has been shown that there are fractionation effects in *Drosophila* spermatids, by Catsch and by Oster.

MULLER: This fractionation effect only exists, perceptibly, at relatively high doses. With *Drosophila*, if you are below 800 r, it is negligible. It is to all extents and purposes linear below 800 r. Above this it begins to take effect. I do think that you could have a fractionation effect on killing, quite apart from any rise above linearity, due to regeneration effects.

HARM: You mentioned the possibility that the two-hit component might be due to the sum of two sub-lethal mutations. Can you test this by further progeny testing, separate them by crossing-over, or so?

TRAUT: I think that Dr. Oster can say something on this point, he knows of a paper on this.

OSTER: I seem to remember that Dr. Hildreth had looked for such synthetic lethals following crossing over between non-irradiated chromosomes from wild populations and that he failed to find them (HILDRETH, P. E., The problem of synthetic lethals in *Drosophila melanogaster*, *Genetics*, **41**, 729, 1956). I might add that in 1957 Dr. Carlson and I had tried to determine whether any of the lethals found by us in an X-ray experiment were in reality due to two (or more) sub-lethal mutations by analyzing the cross-over products derived from females heterozygous for such induced sex-linked lethals. We did not find any in our sample which, unfortunately, was rather small. Hence we did not think it worthwhile to publish the results.

MULLER: It is theoretically necessary that this should occasionally occur, because there has to be a line, for example, 10 per cent viability, arbitrarily dividing lethals from non-lethals. When, therefore, two or more sub-lethals occur together, you will come below this line and it will be counted as a lethal. It has, however, been found that this happens very rarely. I doubt that it would be able to cause much of this effect.

TRAUT: I would claim that our finding of a dose exponent greater than one is justified by the cytological work, where it has been shown that there must be chromosome aberrations requiring at least two hits among the total lethal yield.